



Increment 45/46 Science Symposium

Light Microscopy Module Biophysics -1 (LMM-B1)



Presented by:

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LMM-B1



PI: Dr. Larry DeLucas
University of Alabama at Birmingham

NASA Research Announcement (NRA) - 2013 Macromolecular Biophysics

The Effect of Macromolecular Transport on Microgravity Protein Crystallization

- Experiment to be performed in the FIR LMM.
- Compare incorporation of protein aggregates into growing protein crystals on ISS and on earth
- Measure growth rates in 1g versus microgravity (μg) for different size aggregates of proteins.
- Compare the defect density and crystal quality via fluorescent-based atomic force microscopy and X-ray diffraction quality of crystals grown at different rates in a 1g environment.
- Launches scheduled for **February 2016** on SpaceX-10 (MB1), and **2019** on TBD (B1).

Dr. Larry DeLucas, PI [NASA Team: UAB] PI / Provide flight samples, science requirements, and data analysis	Professor and Director, Center for Structural Biology 1720 2nd Avenue South University of Alabama at Birmingham Birmingham AL. 35294 Tel.: 205-934-5329; delucas@cbse.uab.edu
Dr. Christian Betzel, Co-I	University of Hamburg Tel.: 40-8998 X 4744; Christian.betzel@uni-hamburg.de

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Light Microscopy Module Biophysics -1 (LMM-B1)

- Science Background and Hypothesis
- Investigation goals and objectives
- Measurement approach
- Importance and reason for ISS
- Expected results and how they will advance the field
- Earth benefits/spin-off applications

Science Background and Hypothesis – 1/5

Science Background

There are over 100,000 proteins in the human body and an estimated 10 billion throughout the global environment. To fully understand how they work and how they interact with each other, it is necessary to determine their 3-dimensional structure. This is most often done through analysis of X-ray diffraction of quality crystals.

A newer method, using analysis by neutron diffraction, determines the position of hydrogens within a protein structure and enables more accurate determination of biochemical reactions taking place within and between proteins.

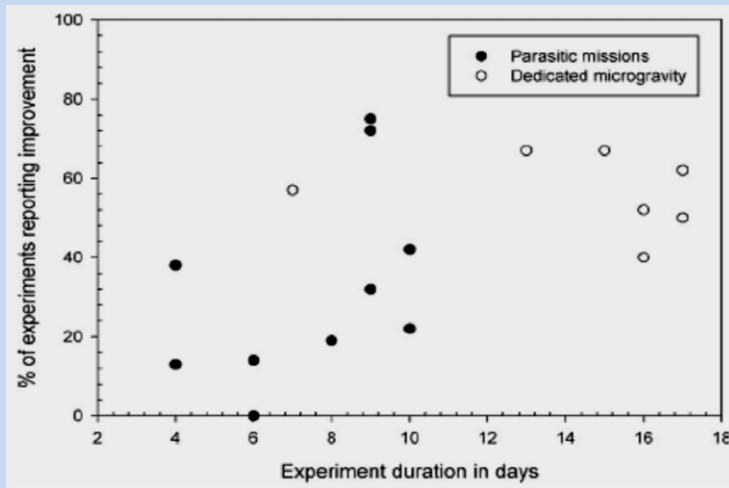
- Neutron diffraction requires very large, quality crystals, greater than 1 millimeter³ in volume.
- Fewer than 100 unique neutron structures of proteins have been reported in the Protein Data Bank, as compared to over 90,000 X-ray diffraction structures.

Quality data for both X-ray diffraction and neutron diffraction structure determination requires crystals of high quality with few defects, and this is often the bottle-neck for crystallographers.

It is particularly difficult to grow high quality crystals of membrane proteins which have the desired qualities. It is estimated that 20 – 30 % of all genes in all genomes are integral membrane proteins and that membrane proteins are the targets of over 50% of all modern medicinal drugs.

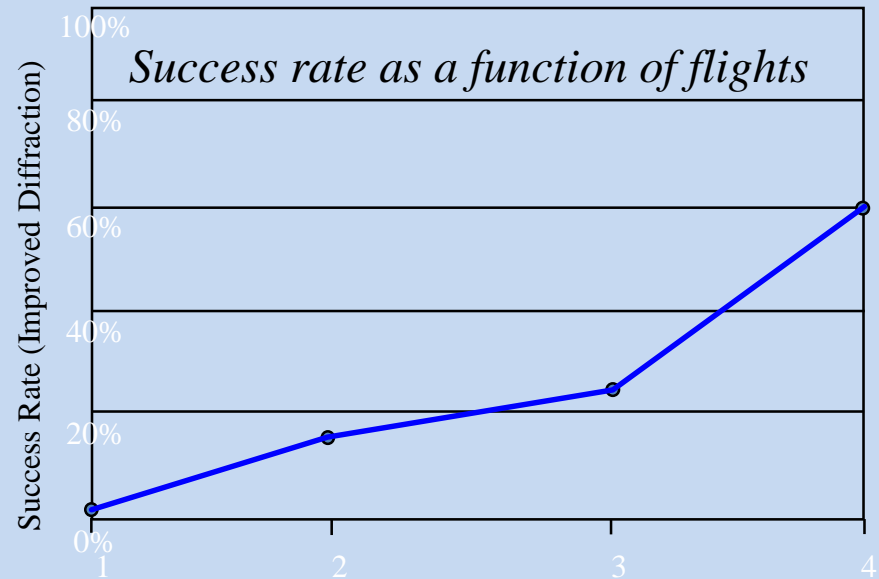
Science Background and Hypothesis – 2/5

Science Background (Cont.)



Protein Crystallization Success Rates versus Mission Duration

Judge, R.A., et. al., "Extracting Trends from Two Decades of Microgravity Macromolecular Crystallization History", (2005), *Acta Cryst. D*, p. 763-771.



Kundrot, C.E., et. al., "Microgravity and Macromolecular Crystallography", (2001), *Crystal Growth and Design*, vol 1, No. 1, p. 87-99.

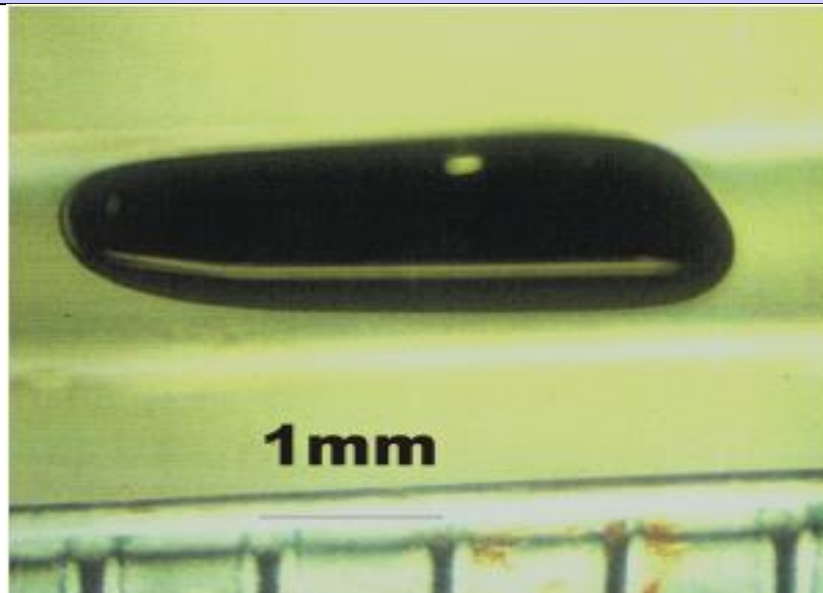
Review Publication: 63 Space Shuttle flights by more than 150 investigators from the international scientific community (Kundrot, C.E., et. al., "Microgravity and Macromolecular Crystallography", (2001), *Crystal Growth and Design*, vol 1, No. 1, p. 87-99):

- 179 proteins flown prior to STS-95, 36 proteins (20%) obtained the highest diffraction resolution to date from the microgravity-grown crystals.
- Direct correlation between success rates and the number of times a protein is flown
 - Of 81 proteins that flew only once, only two showed increased diffraction quality. In contrast, more than 50% of the proteins that had four flight opportunities produced crystals with increased diffraction quality.
- The magnitude of the diffraction improvement varied significantly but in more than 50% of the cases, the microgravity-grown crystals resulted in at least a doubling of the amount of diffraction data.

Science Background and Hypothesis – 3/5

Science Background (cont.)

“The crystals grown under microgravity were up to 20 times larger than all crystals grown on earth previously. The largest microgravity-grown crystal was 4mm long and 1.5 mm in diameter. The native data collected from a microgravity-grown crystal formed the basis for the improved crystal structure of PSI at 4 angstroms resolution”



Large single crystal of photosystem I,
grown in the space shuttle Columbia
during the USML-2 Mission

Science Background and Hypothesis – 4/5

Science Background (cont.)

How Does Microgravity Influence Protein Crystallization?

Elimination of sedimentation and convection can enhance protein crystallization.

During the nucleation event, the solution in the immediate vicinity of the growing crystal becomes depleted of protein molecules as the crystal nucleates and begins to grow.

- Therefore the local supersaturation on the surface of the growing crystal is decreased, favoring crystal growth over additional nucleation. In other words, the growth of pre-existing crystals predominates over the initiation of new crystal formation, resulting in bigger and fewer crystals.

When a new protein molecule aligns itself on the surface of a growing crystal, its movement through the solution toward the growing crystal is dictated by diffusion rather than by buoyancy-induced convective sources.

- Therefore, protein molecules traverse the droplet at a very slow rate providing more time for crystal lattice alignment thus producing a higher quality crystal.

Science Background and Hypothesis – 5/5

Hypothesis

- Protein crystals have been grown in microgravity for over 3 decades. Over 125 research publications and review articles provide compelling evidence of the beneficial effect of a microgravity environment for protein crystallization.
- There is still a lack of understanding as to why microgravity is beneficial to protein crystallization. This project will investigate the influence of two theories proposed by the scientific community to explain the beneficial influence of microgravity on protein crystallization.

Hypothesis

Improved quality of microgravity-grown protein crystals is the result of two macromolecular characteristics that exist in a buoyancy-free, diffusion-dominated solution:

1. *Slower crystal growth rates, due to slower protein transport to the growing crystal surface*
2. *Predilection of growing crystals to incorporate protein monomers versus higher protein aggregates due to differences in transport rates*

LMM-B1 Investigation Goals and Objectives

Aim-1: In μg and concurrently in 1g control experiments, compare incorporation of protein aggregates (monomers versus dimers, trimers, etc.) into growing protein crystals for proteins with molecular weights ranging from less than 20kDa to 1,200kDa or more.

Detection of aggregates in the crystal will be accomplished via LMM's fluorescence microscope for the first flight, with the addition of confocal capabilities for the second flight.

Based on the percentage incorporation of larger aggregates within the crystal, the effect of molecular filtering based on differences in diffusion rates will be assessed.

Aim-2: Measure crystal growth rates in 1g versus μg for proteins with molecular weights ranging from less than 20kDa to more than 1,200kDa.

Aim-3: Compare the defect density and crystal quality of crystals grown at different rates in a 1g environment (analysis by atomic force microscopy and x-ray diffraction studies).

For this aim a specific crystallization system (*Xtal Controller* made available via collaboration with Dr. Christian Betzel, University of Hamburg) will be used to control the nucleation and subsequent growth rates of the protein crystals.

Results from these studies will be used to determine optimum 1g crystallization conditions. These identical conditions will be evaluated in μg for each protein.

LMM-B1 Measurement approach

We will be using a flight-hardened Commercial-Off-The-Shelf (COTS) microscope

[pictured on next page]

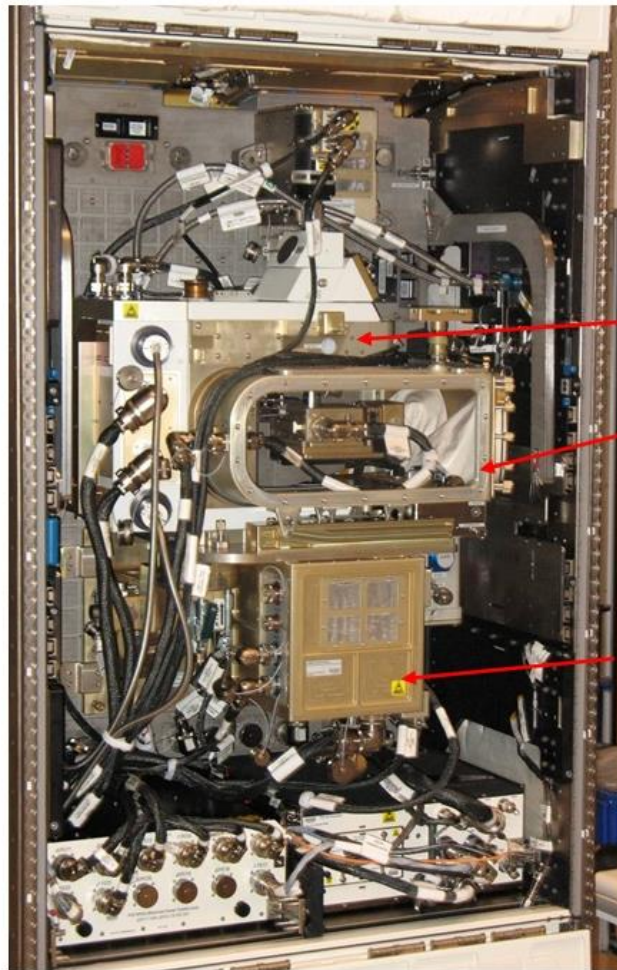
and a

Macromolecular Biophysics sample module

[pictured later]

Measurement approach – 1/4

Light Microscopy Module (LMM) in the Fluid Integrated Rack (FIR)



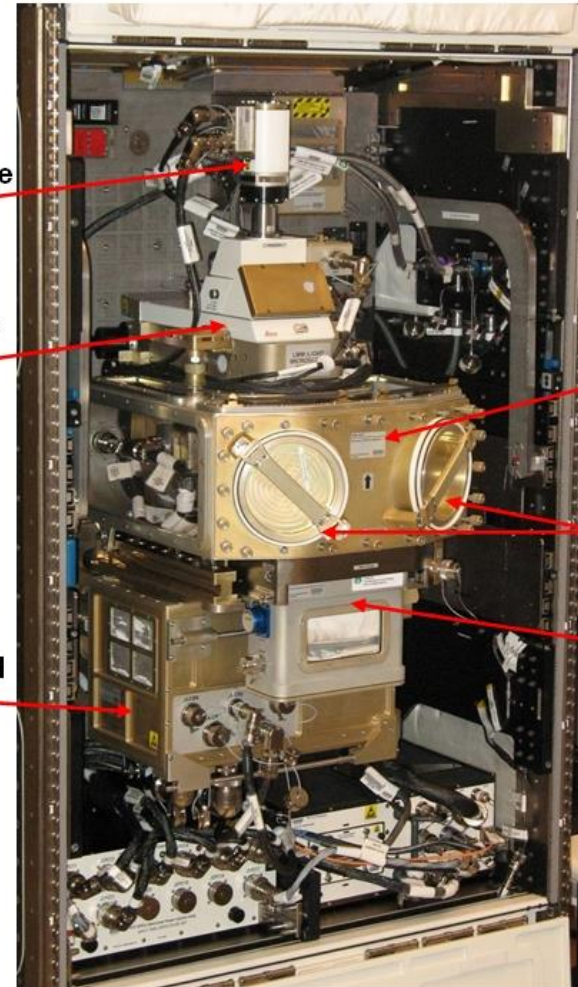
LMM in the Closed Position or Operating Configuration

Monochrome Camera

Microscope

Auxiliary Fluids Container (AFC) – Left Side

LMM Control Box



LMM in the Open Position or Installation/Service Configuration

Auxiliary Fluids Container AFC - Front

Glove ports

Equipment Transfer Module (ETM)

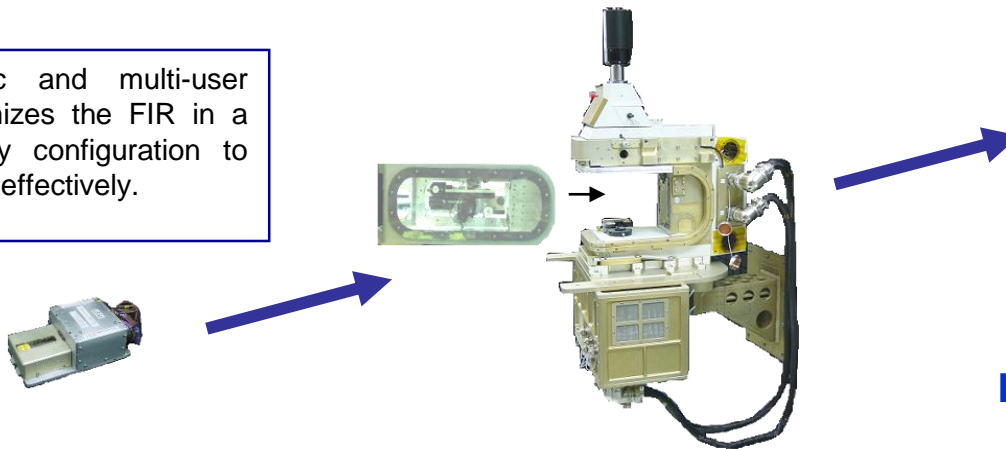
Measurement approach – 2/4

LMM Implementation Philosophy

Philosophy: Maximize the scientific results by utilizing the existing LMM capabilities. Develop small sample modules and image them within the LMM

Payload specific and multi-user hardware customizes the FIR in a unique laboratory configuration to perform research effectively.

Light Microscopy Module



Payload Specific Hardware

- Sample Cell with universal Sample Tray
- Specific Diagnostics
- Specific Imaging
- Fluid Containment

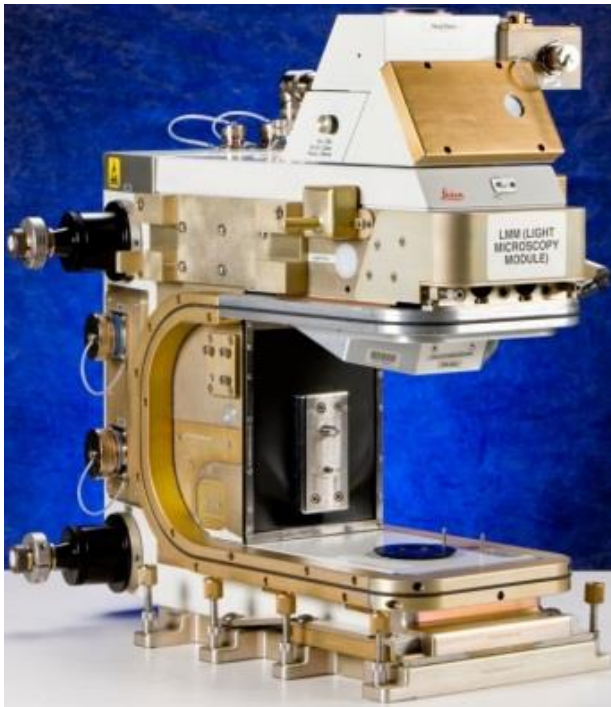
Multi-Use Payload Apparatus

- Test Specific Module
- Infrastructure that uniquely meets the needs of PI experiments
- Unique Diagnostics
- Specialized Imaging
- Fluid Containment

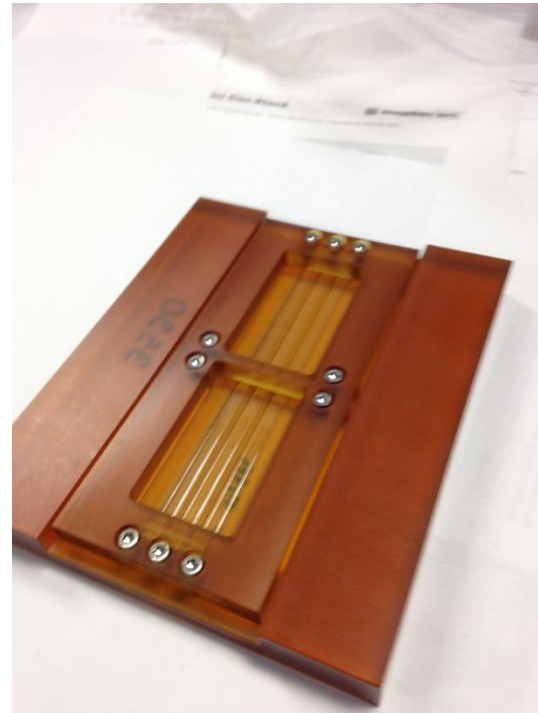
FCF Fluids Integrated Rack

- Power Supply
- Avionics/Control
- Common Illumination
- PI Integration Optics Bench
- Imaging and Frame Capture
- Diagnostics
- Environmental Control
- Data Processing/Storage
- Light Containment
- Active Rack Isolation System (ARIS)

LMM-B1 Measurement approach – 3/4

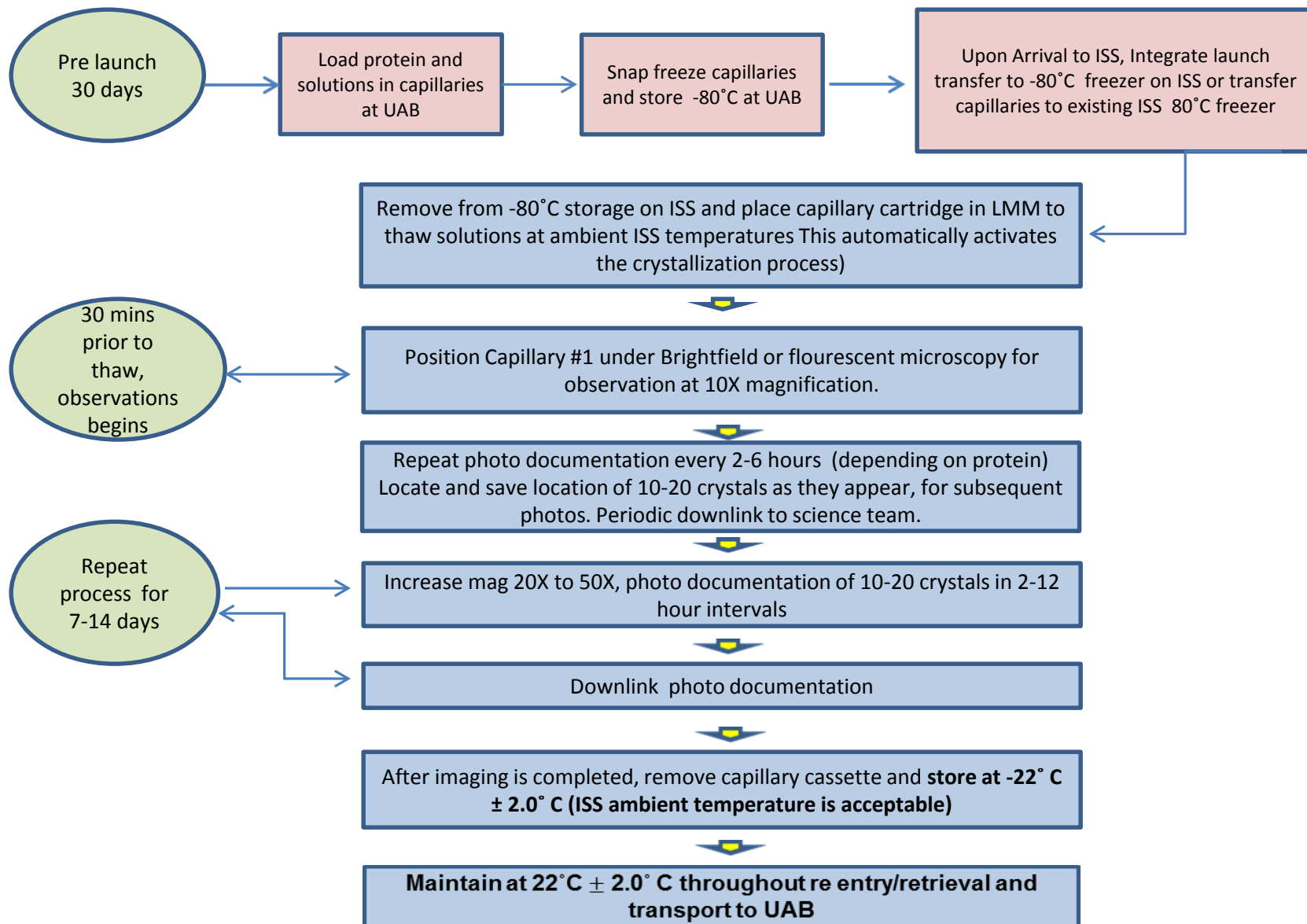


Light Microscopy Module
(LMM)



LMM-B1 Sample Assembly
Prototype that will contain up
to 8 square 100mm capillaries

Experimental Procedure for Capillary Observation and Photo Documentation on ISS



There are more than 125 research publications and review articles that provide compelling evidence of the beneficial effect of a microgravity environment for protein crystallization.

- However, many scientists and in particular, crystallographers, remains skeptical. Why?
- a) Due to short μg duration, crystals for a protein flown for an investigator were too small to support diffraction studies (crystal growth is slower in μg)
 - b) Shuttle flight was delayed resulting in protein degradation prior to flight
 - c) Solutions did not contain any crystals (nucleation is dramatically slower in μg)
 - d) Protein batch prepared for mission simply was not as good as other batches used for earth studies
 - e) Experiments lacked proper control experiments or results were not statistically convincing
 - f) ***Lacking a comprehensive understanding as to why microgravity is beneficial to protein crystallization.***

➤ ***This proposal will investigate the influence of two theories proposed by the scientific community to explain the beneficial influence of microgravity on protein crystallization.***

Expected results and how they will advance the field

Long-duration protein crystal growth experiments on the ISS with photo documentation, and subsequent analysis of the comparisons between 1g and μg crystals, will enable a more complete understanding of why proteins and other macromolecules often form more perfect crystals in microgravity than they do on earth.

Earth benefits / spin-off applications

- In spite of major advances in the rate of crystallizing novel proteins there are more than 9,000 previously crystallized human aqueous and membrane proteins, targeted by NIH as extremely important for structure determination, for which the crystals are of insufficient quality to yield a structural solution (based on NIH Structural Genomics websites).
- Structural biology of protein-protein complexes and integral membrane proteins are currently a high NIH priority due to their importance for systems biology, disease mechanisms and structure-guided drug development.



LMM-B1

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BACKUP SLIDES

Mission Success Criteria for LMM-B1 (DeLucas)

Success Level	Accomplishment
Minimum Success	(1) At the completion of both flights, for one of the 6 different protein/virus samples: must be able to accurately compare crystal growth rates in microgravity versus 1g (this specific aim utilizes LMM's bright field microscopy capability) (2) For one of the 6 different protein/virus samples: must be able to compare the approximate percentage incorporation of higher macromolecular aggregates in crystals grown in microgravity versus 1g (this specific aim utilizes LMM's planned the confocal fluorescent microscopy capability)
Significant Success	(1) At the completion of both flights, for 4 of the 6 different protein/virus samples: must be able to accurately compare crystal growth rates in microgravity versus 1g (this specific aim utilizes LMM's bright field microscopy capability) (2) For 4 of the 6 different protein/virus samples: must be able to compare the approximate percentage incorporation of higher macromolecular aggregates in crystals grown in microgravity versus 1g (this specific aim utilizes LMM's planned the confocal fluorescent microscopy capability)
Complete Success	(1) At the completion of both flights, for all of the 6 different protein/virus samples: must be able to accurately compare crystal growth rates in microgravity versus 1g (this specific aim utilizes LMM's bright field microscopy capability) (2) For all of the 6 different protein/virus samples: must be able to compare the approximate percentage incorporation of higher macromolecular aggregates in crystals grown in microgravity versus 1g (this specific aim utilizes LMM's planned the confocal fluorescent microscopy capability)